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ORIGINAL ARTICLE

Long-term outcome for Down syndrome patients with hematopoietic disorders



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Received 26 September 2014; received in revised form 4 January 2015; accepted 22 January 2015

KEYWORDS

acute myeloid
leukemia;
Down syndrome;
GATA1;
myeloproliferative
disorders;
transcription factor

Background/purpose: Although Down syndrome (DS) patients have a higher risk of developing transient myeloproliferative disorder (TMD) and acute leukemia, very little data is available on long-term outcome in Taiwanese patients. The current study was designed to determine the clinical characteristics and treatment outcome of DS patients with TMD or acute leukemia (AL).

Methods: In 25 consecutive DS patients with TMD or AL enrolled from 1990 to 2012, clinical manifestations and treatment protocols were investigated and GATA1 (GATA binding protein 1) mutations were identified. Among 16 DS-acute myeloid leukemia (DS-AML) patients, clinical outcomes were compared between survivors and nonsurvivors.

Results: Most of our DS patients had TMD (32%), acute megakaryoblastic leukemia (24%), or acute erythromegakaryoblastic leukemia (16%). The median follow-up time was 22.5 months (1–230 months). The age was younger and the hemoglobin (Hb) level and platelet count were higher in TMD patients than in leukemia patients. Among DS-AML patients, the Hb level was higher in survivors than nonsurvivors (8.8 ± 2.7 g/dL vs. 5.8 ± 2.4 g/dL; $p = 0.044$) and the age was older in relapsed patients than in nonrelapsed patients (43.8 ± 18 months old vs. 21.6 ± 8.6 months old; $p = 0.025$). The 3-year overall survival (OS) rate was 44%, higher in patients receiving appropriate chemotherapy than in those receiving inadequate treatment

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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<http://dx.doi.org/10.1016/j.jfma.2015.01.009>

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(63.6% vs. 0%, $p = 0.001$), and higher in those diagnosed with TMD or AL after 2008 than before 2008 (33.3% vs. 75%; $p = 0.119$).

Conclusion: Outcome in DS-AML patients is optimal if appropriate treatment is provided. With modification of the treatment strategy in 2008, OS increased in Taiwan.

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Introduction

Trisomy 21 [Down syndrome (DS); MIM #190685; a common chromosome aneuploidy] is associated with an increased risk of early-onset hematopoietic disorders.¹ Around 10% of newborns with DS develop transient myeloproliferative disorder (TMD).² DS children have a 50-fold increased incidence of acute leukemia during the first 5 years of life.^{3,4} The acute leukemias in approximately 60% of affected DS children are myeloid, with at least 50% of these being acute megakaryoblastic leukemia (AMKL).⁵ Mutations in exon 2 of the *GATA1* (GATA binding protein 1) gene, resulting in a premature stop codon within the N-terminal activation domain (i.e., GATA1s, a truncated form of GATA1) have been detected in almost all TMD and DS AMKL cases.^{6–9} Both GATA1s and GATA1 have similar DNA binding abilities and interact with partner proteins, such as “Friend of GATA1” (FOG1).¹⁰ Expression of this mutated form potentially contributes to the uncontrolled proliferation of poorly differentiated megakaryocytic precursors.¹¹

Acute myeloid leukemia (AML) in patients with DS is referred to as myeloid leukemia associated with DS (DS-AML) in the 4th edition of the World Health Organization classification.⁴ The overall survival (OS) of DS-AML patients is approximately 80%.^{12–19} However, more accurate pre-natal screening in Taiwan in recent years has led to fewer DS births, and fewer DS-TMD or DS-acute leukemia (DS-AL) cases are available for study. In general, the treatment protocols for DS-AL patients are based on the recommendations of the Taiwan Pediatric Oncology Group (TPOG). Because reports of clinical presentations or long-term outcome in the early period are sporadic and few in number,^{20–23} the present study aimed to investigate the long-term outcome in Taiwanese patients with DS-TMD or DS-AL and to determine the risk factors for DS-TMD or DS-AL.

Methods

Patients and sample collection

We retrospectively reviewed the medical records of 25 consecutive DS patients with hematopoietic disorders diagnosed in our hospital (or whose samples were sent to our hospital for *GATA1* mutation analysis) from 1990 to 2012. DS was diagnosed shortly after birth using karyotype analysis and clinical manifestations. TMD was diagnosed before the age of 3 months by the presence of peripheral blood nonerythroid blasts and by reduction in the peripheral blood blast cell percentage occurring in response to

cytoreductive therapy or spontaneously.²⁴ Acute leukemia was diagnosed by morphologic, biochemical, immunophenotypic, and cytogenetic analysis of bone marrow or peripheral blood and classified according to French–American–British (FAB) criteria.²⁵ Age at onset, sex, initial white blood cell count, blast percentage, hemoglobin (Hb) level, platelet count, levels of alanine transaminase, aspartate transaminase, lactate dehydrogenase, and uric acid, karyotype results, immunophenotyping results, treatment regimens, and outcomes were all retrospectively obtained from the charts. Study protocol approval was obtained from our institutional ethics committee (No. 201312034RINC).

Chemotherapy

The treatment protocols for DS with AL are based on the recommendations of the TPOG and include the standard protocol for acute lymphoblastic leukemia,²⁶ and several modifications of the standard protocol for *de novo* AML (i.e., the TPOG AML 901 protocol, AML 97A protocol, and AML 97B protocol before and during 2008 and the TPOG AML-DS 2008 protocol after 2008).²⁷ The protocol-specified treatment periods were approximately 1 year.

The TPOG AML 901 protocol used since 1990 consisted of three steps: induction [i.e., treatment with epirubicin, cytosine arabinoside (Ara-C), and 6-thioguanine (6-TG)]; consolidation (i.e., treatment with etoposide and cyclophosphamide), and maintenance [i.e., treatment with epirubicin, Ara-C, 6-TG, etoposide, cyclophosphamide, mercaptopurine, and methotrexate (MTX)]. The AML 901 protocol was revised in 1997 and the resulting AML 97A and AML 97B protocols differed from their predecessor as follows. The AML 97B protocol consisted of the same three steps but used idarubicin instead of epirubicin and added mitoxantrone. The AML 97A protocol consisted of only two steps: induction with Ara-C and idarubicin, and post-remission with high-dose Ara-C (1 g/m²), etoposide, mitoxantrone, and idarubicin. The DS 2008 protocol used the half dose regimen of the AML 97A protocol but reduced the Ara-C dose by 25%, added intrathecal MTX to the induction regimen, and used L-asparaginase instead of mitoxantrone in the AML 97A consolidation regimen. In our study, use of either the full- or half-dose AML 901, AML 97A, or AML 97B protocols and the AML DS 2008 protocol in DS-AML patients was regarded as *standard treatment*. The use of palliative or partial treatment was regarded as *incomplete treatment*. Our patients were followed up from January 1990 to June 2014 inclusively. The duration of OS was measured from the time of diagnosis to the date of death or last follow up.

GATA1 mutation analysis

Genomic DNA was isolated from peripheral blood leukocytes or bone marrow aspirates using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). GATA1 exon 2 coding regions and their flanking intronic sequences were amplified using polymerase chain reaction (PCR) (primer sequences and protocol available in Table S1). For those samples found to be negative for exon 2 mutation, the whole GATA1 gene was sequenced. The PCR products were purified using the Gel-M Gel Extraction System (Viogene, Taipei, Taiwan) and analyzed by direct sequencing using the ABI Prism Big Dye dideoxy chain terminator cycle sequencing kit and the ABI Prism 310 genetic analyzer (Applied Biosystems, Bedford, MA, USA). The nucleotides of the GATA1 complementary DNA were numbered starting from the translation initiation site (NM_000023.10). Samples of 13 DS patients without TMD or AL (DS without leukemia) and two AMKL patients without DS were also analyzed for comparison.

Statistical analysis

Statistical analyses were performed using SPSS statistical package, version 11.5 (SPSS Inc., Chicago, IL, USA). The Student *t* test or Mann-Whitney rank test was used for direct comparisons between groups. Data are presented as the mean \pm standard deviation. OS percentages and standard errors were calculated using the Kaplan-Meier method, and log-rank tests were used for group comparisons. A *p* value < 0.05 was considered statistically significant.

Results

Clinical presentations and outcomes

The patients' clinical characteristics are detailed in Table S2. Most DS patients had TMD ($n = 8$), AMKL ($n = 6$), or acute erythromegakaryoblastic leukemia (FAB M6/M7, mixed lineage; $n = 4$; Fig. 1). Compared with DS-AL patients, DS-TMD patients were younger (0.34 ± 0.46 months old vs. 29.1 ± 15.7 months old; $p < 0.001$), had a higher Hb level (12.7 ± 4.4 g/dL vs. 6.9 ± 2.7 g/dL; $p < 0.001$) and higher platelet level (165 ± 223 k/ μ L vs. 35.9 ± 35.1 k/ μ L; $p = 0.026$). Other parameters were indistinguishable (Table 1).

The OS rate in 16 DS-AML patients was 31% and increased after the year 2008, although not significantly (17% vs 75%; $p = 0.063$). Survivors had a lower relapse rate (0% vs. 45%; $p = 0.013$) and higher Hb level (8.8 ± 2.7 g/dL vs. 5.8 ± 2.4 g/dL; $p = 0.044$) than nonsurvivors (Table 2). The survival outcome was better among those receiving standard treatment than those receiving incomplete treatment (median survival 48.5 months vs. 3 months, $p = 0.0013$; Fig. 2). Five patients (31%) relapsed, including three treated with the AML 97A half-dose protocol, one with the AML 97B protocol, and one with the DS 2008 protocol. The median survival time was significantly worse among relapsed patients than nonrelapsed patients (18.2 months

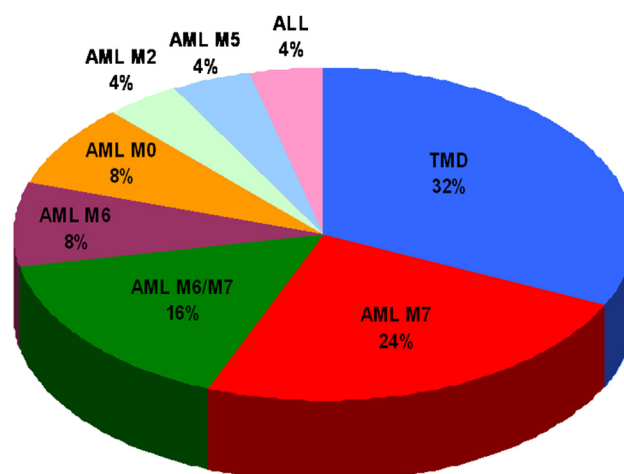


Figure 1 Percentage of patients with each disease. Most patients had transient myeloproliferative disorder (TMD; 32%), acute megakaryoblastic leukemia (AML, M7; 24%), or acute erythromegakaryoblastic leukemia (AML, M6/M7; 16%).

Table 1 Comparison of clinical characteristics between TMD and DS-AL patients.

	TMD	DS-AL	<i>p</i>
Sex (M/F)	3/5	10/7	0.286
Age (mo)	0.34 ± 0.46	29.1 ± 15.7	< 0.001
WBC count ($10^3/\mu$ L)	104 ± 146	28 ± 48	0.061
Hb (g/dL)	12.7 ± 4.4	6.9 ± 2.7	< 0.001
Platelet count ($10^3/\mu$ L)	165 ± 223	35.9 ± 35.1	0.026
ALT (U/L)	84 ± 90	135 ± 270	0.726
AST (U/L)	89 ± 55	82 ± 85	0.880
LDH (U/L)	3338 ± 1874	2025 ± 2172	0.319
UA (mg/dL)	6.9 ± 5.4	5.5 ± 1.5	0.420

ALT = alanine transaminase level; AST = aspartate transaminase level; DS-AL = Down syndrome–acute leukemia; F = female; Hb = hemoglobin level; LDH = lactate dehydrogenase level; M = male; TMD = transient myeloproliferative disorder; UA = uric acid level; WBC = white blood cell.

Table 2 Comparison between survivors and nonsurvivors in DS-AML patients.

	Died ($n = 11$)	Survived ($n = 5$)	<i>p</i>
Sex (M/F)	6/5	3/2	0.635
Age (mo)	31.6 ± 16.9	20 ± 8.6	0.174
Relapsed	5 (45%)	0 (0%)	0.013
WBC count ($10^3/\mu$ L)	31 ± 53	28 ± 47	0.938
Hb (g/dL)	5.8 ± 2.4	8.8 ± 2.7	0.044
Platelet count ($10^3/\mu$ L)	29 ± 26	49 ± 53	0.316

DS-AML = Down syndrome–acute myeloid leukemia; F = female; Hb = hemoglobin level; M = male; WBC = white blood cell.

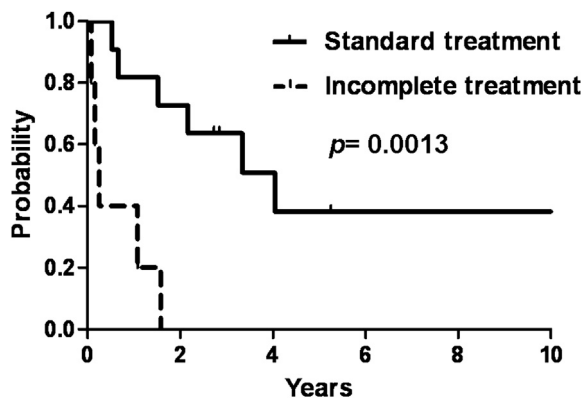


Figure 2 Ten-year overall survival (OS). Outcome was better in patients receiving standard treatment (vs. incomplete treatment; $p = 0.0013$).

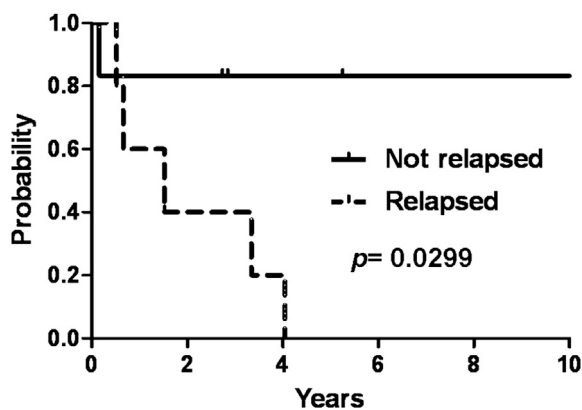


Figure 3 Ten-year overall survival (OS). Outcome was worse in relapsed patients (vs. nonrelapsed; $p = 0.0299$).

vs. not reached at 120 months; $p = 0.0299$; Fig. 3), and age was significantly older among relapsed patients than non-relapsed patients (43.8 ± 18 months old vs. 21.6 ± 8.6 months old; $p = 0.025$).

GATA1 mutation analysis

GATA1 mutation was investigated in 11 patients with DS-TMD or DS-AL and in DNA extracted from either bone marrow (in 2 DS-TMD patients and all DS-AL patients) or peripheral blood (in 1 DS-TMD patient). *GATA1* gene mutation was detected in 90% of patients tested (i.e., 3 of 3 DS-TMD patients and 7 of 8 DS-AL patients). All *GATA1* mutations were novel mutations clustering in exon 2 (Table 3). Eight mutations [7 premature termination codon (PTC) type 1–3 mutations and 1 PTC type 1–5 mutation] resulted in premature termination of translation and two mutations resulted in splicing errors. No *GATA1* mutation was detected in DS patients without leukemia ($n = 13$) and non-DS patients with AMKL ($n = 2$; Table 4). Of the 10 patients with *GATA1* mutation, six patients (60%) survived with remission. The one patient who lacked a detectable *GATA1* mutation relapsed and died.

Discussion

This study analyzed the clinical long-term outcomes of 25 DS patients with hematopoietic disorders. The OS was 40%. For DS-AML patients, outcome was better in patients who received standard chemotherapy treatment, had a higher initial Hb level, and had not relapsed.

The OS rate was quite low and 10 of 17 (58.8%) DS-AL patients died. However, only one patient died after the year 2008 (due to relapse). Familial support plays a role in compliance with chemotherapy. In the period before 2008, most mortality was due to factors affecting relapse such as the negative attitudes of family members and consequently their provision of relatively poor supportive care. After year 2008, the revised treatment protocol and more aggressive supportive care provided by parents increased the survival rate. Nevertheless, the 3-year OS rate among Down syndrome patients with relapsed acute leukemia remains low (i.e., only 25.9% in the study by Taga et al²⁸ and 40% in our study). Taga et al²⁸ identified a long interval between initial diagnosis and relapse as a significant favorable prognostic factor. Although we did not identify this factor, possibly due to the small sample size, we did notice that relapsed patients were older. We supposed that disease in older DS-AML patients was of a distinct subtype that was relatively more resistant to therapy. These patients either failed to achieve remission or eventually relapsed after treatment. In previous reports, outcomes were worse and treatment requirements more dose-intensive in children with DS than in those without DS.^{9,19,29} Taga et al²⁸ also noted that second-line chemotherapy was less likely to achieve complete remission in patients who relapsed earlier.

The *GATA1* mutation rate in our study was 90%, which is similar to previously reported mutation rates of 97.3% in DS-TMD and around 89.2% in DS with acute megakaryoblastic leukemia.⁸ Until now, >100 *GATA1* mutations have been reported, mostly occurring in exon 2 and causing protein truncation.² Our mutations were also located in exon 2 or intron 2, suggesting that this region is a mutagenesis hotspot.

The Hb level was higher in survivors than nonsurvivors (8.8 ± 2.7 g/dL vs. 5.8 ± 2.4 g/dL; $p = 0.044$), higher in DS-TMD than in DS-AL patients, and may indicate better overall health and health benefits, such as lower leukemia burden, less bleeding tendency, and better oxygen supply. Although treatment prognosis is multifactorial, a higher Hb level appeared to be associated with better outcome. Further studies are needed to confirm this hypothesis.

The major limitation of this study is the relatively small sample size. It would have been more valuable to conduct a multicenter, prospective cohort study over the same period of time. Nonetheless, this study is the first in the literature to provide a large-scale retrospective clinical and molecular assessment of DS patients with hematopoietic disorders in the Chinese population. Also, because the study was conducted over a long period, we were able to follow treatment outcome as treatment strategies evolved. Before the era of the DS-AML specific protocol, physicians in Taiwan most often used a modified TPOG AML protocol. However, the outcome of treatment (because of variation in drug dose and frequency) was less than optimal. The DS 2008 protocol incorporated the half-dose regimen used in

Table 3 GATA1 mutation analysis.

Patient	Diagnosis	Location	GATA1 mutation	Consequence of mutation	Mutation type
2	TMD	Intron 2	del6bp from IVS2+1	Splice mutant	Splicing error
5	TMD	Exon 2	183 ins CC	Frameshift at codon 61, stop at codon 198	PTC 1–3'
8	TMD > AML M7	Exon 2	194–267 del 74 bp	Frameshift at codon 65, stop at codon 408	PTC 1–3'
13	AML, M6	Exon 2	139 ins C	Frameshift at codon 47, stop at codon 114	PTC 1–3'
16	AML, M6/7	Exon 2	87 ins 55 bp	Frameshift at codon 29, stop at codon 57	PTC 1–5'
17	AML, M6/7	Exon 2	192 ins 22 bp	Frameshift at codon 62, stop at codon 207	PTC 1–3'
18	AML, M6/7	Exon 2	157 ins GAGCACA	Frameshift at codon 53, stop at codon 70	PTC1–3'
20	AML, M7	Exon 2	150 del 27 bp ins TACT	Frameshift at codon 51, stop at codon 59	PTC 1–3'
22	AML, M7	Intron 2	IVS2-1G > C	Splice mutant	Splicing error
23	AML, M7	—	No mutation	—	—
24	AML, M7	Exon 2	189 ins GCCTACTA	Tyr63 stop	PTC 1–3'

AML = acute myeloid leukemia; GATA1 = GATA binding protein 1; PTC = premature termination codon; TMD = transient myeloproliferative disorder.

Table 4 GATA1 mutation analysis in TMD, DS-AML, AMKL without DS, and DS without leukemia.

Sample origin	TMD (n = 3)	DS-AML (n = 9)	AMKL without DS (n = 2)	DS without leukemia (n = 13)
Bone marrow	2/2 (100%)	5/6 (83.3%)	0/2	0/0
Peripheral blood	1/1 (100%)	2/3	0/0	0/13

AMKL = acute megakaryoblastic leukemia; DS = Down syndrome; AML = Down syndrome–acute myeloid leukemia; GATA1 = GATA binding protein 1; TMD = transient myeloproliferative disorder.

the AML 97A protocol but reduced the Ara-C dose by 25%, added intrathecal MTX to the induction regimen, and replaced L-asparaginase with mitoxantrone in the consolidation regimen. The TPOG reported that, as a result, the OS rate improved to 92% (unpublished). Thus, in DS-AL patients, less intensive chemotherapy and better supportive care produces the best outcome, thereby giving credence to the use of this strategy in such patients.

In conclusion, the outcome of DS patients with AML when treated appropriately is optimal. Supportive care improvement and treatment protocol modification increased the OS rate in Taiwan after the year 2008. Relapse remains a problem for DS-AML patients and a further larger-scale study is warranted.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.jfma.2015.01.009>.

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